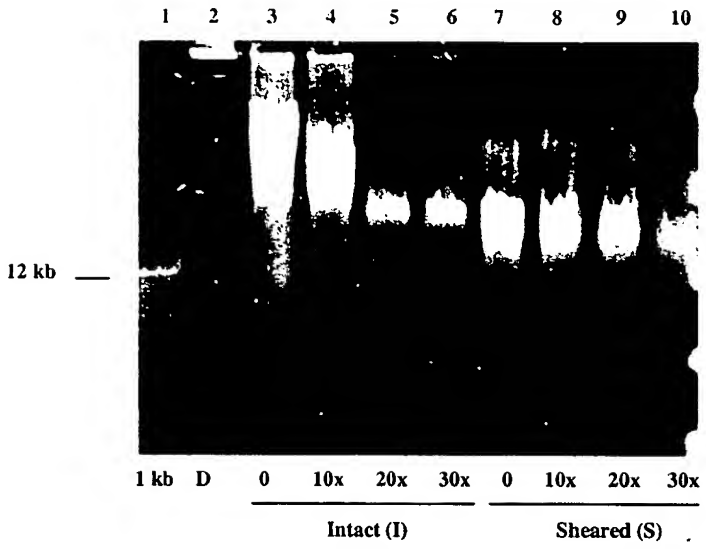
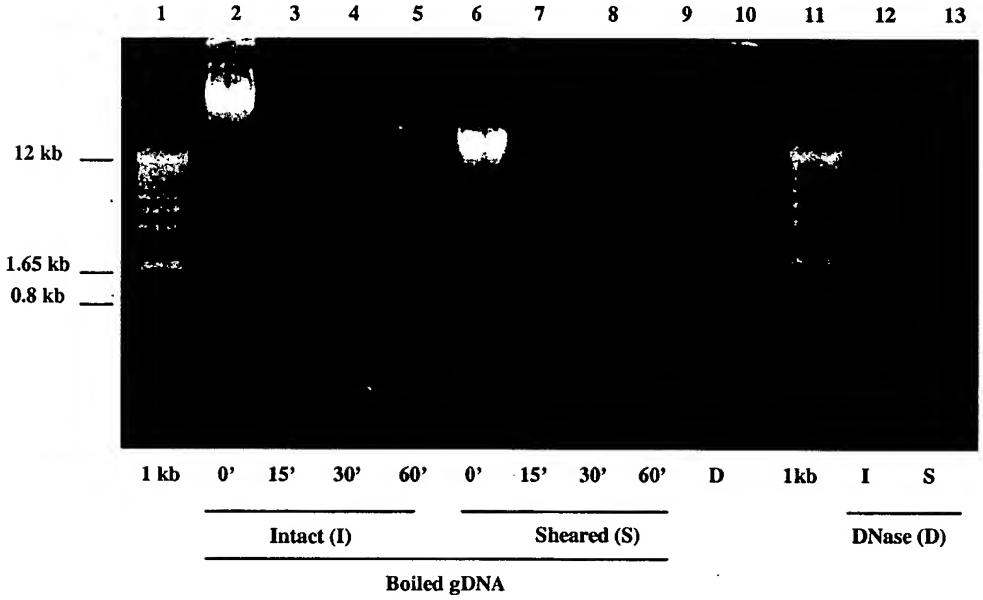
Mechanical Shearing of CEPH 1347-2 gDNA using the TECAN



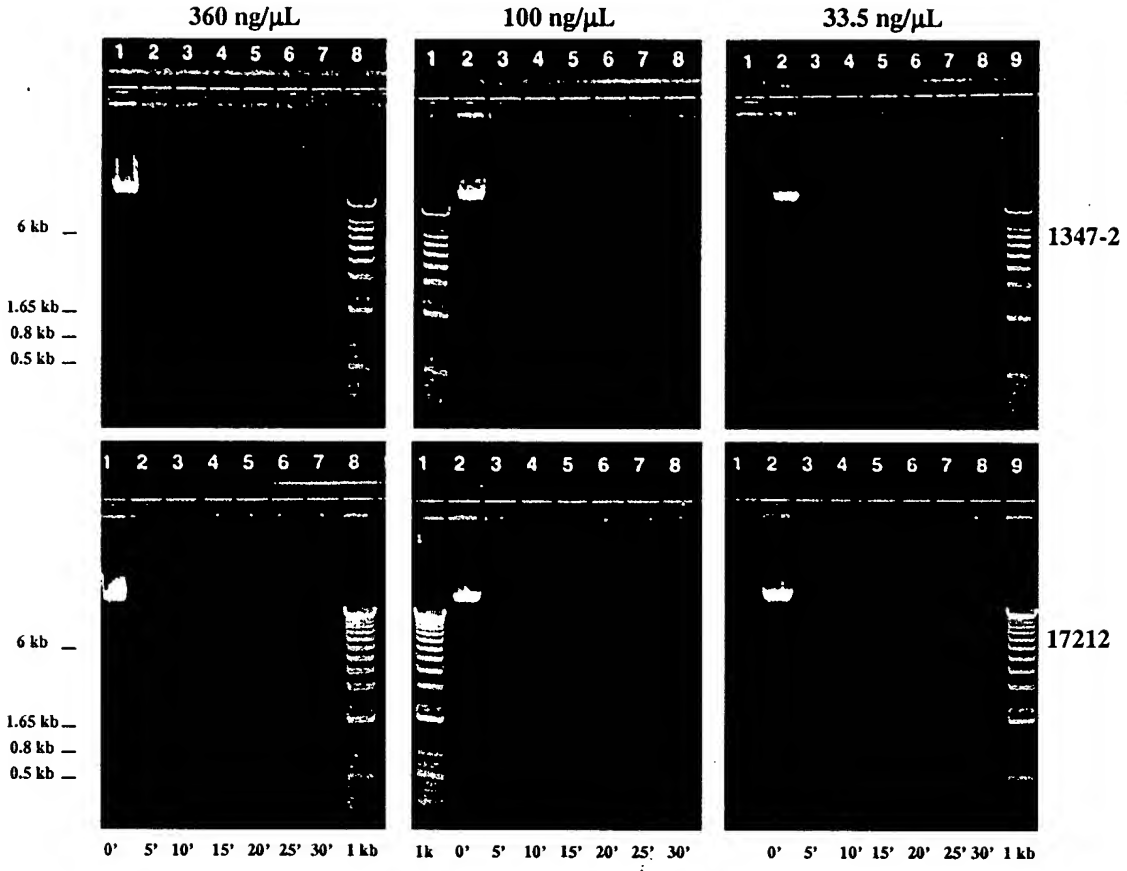
Boiling or DNase I-digestion of CEPH 1347-2 gDNA



CEPH 1347-2 gDNA, 100 ng/μL in 1x TE, pH 7.4 was either mechanically sheared, boiled for the indicated times, or treated with DNase I under standard conditions. 1 μg/lane was subsequently loaded onto 0.5% agarose gels containing 1x Tris-Borate-EDTA, pH 8.

Figure 1

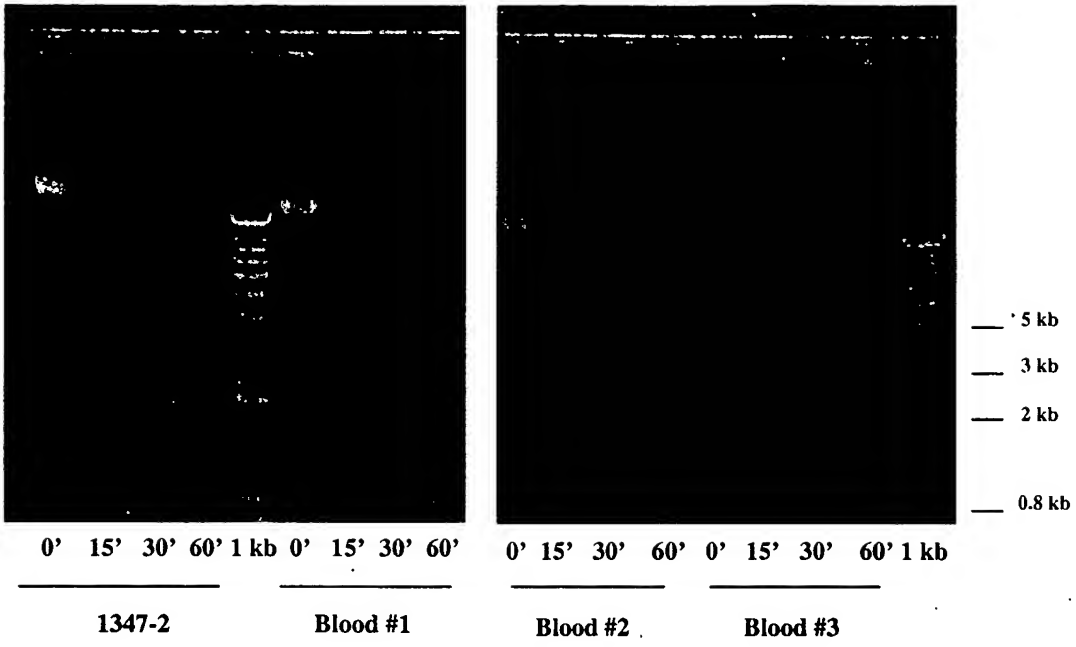
Effect of gDNA Concentration and Boiling Duration on Range of Fragment Size Generated



CEPH 1347-2 or NA-17212 gDNA (33.5-360 ng/µL in 1x TE, pH 8) was boiled for the indicated times, then 0.5 µg of the boiled sample was loaded onto 0.8% agarose E-gel and subjected to electrophoresis.

Figure 2

Fragment Sizes Generated by Boiling of Different Sources of gDNA



CEPH 1347-2 gDNA or gDNA derived from 3 different blood donors, 100 ng/µL in 1x TE, pH 8 was boiled, and 0.5 µg/sample was subsequently loaded onto 0.8% agarose E-gels and subjected to electrophoresis.

Figure 3

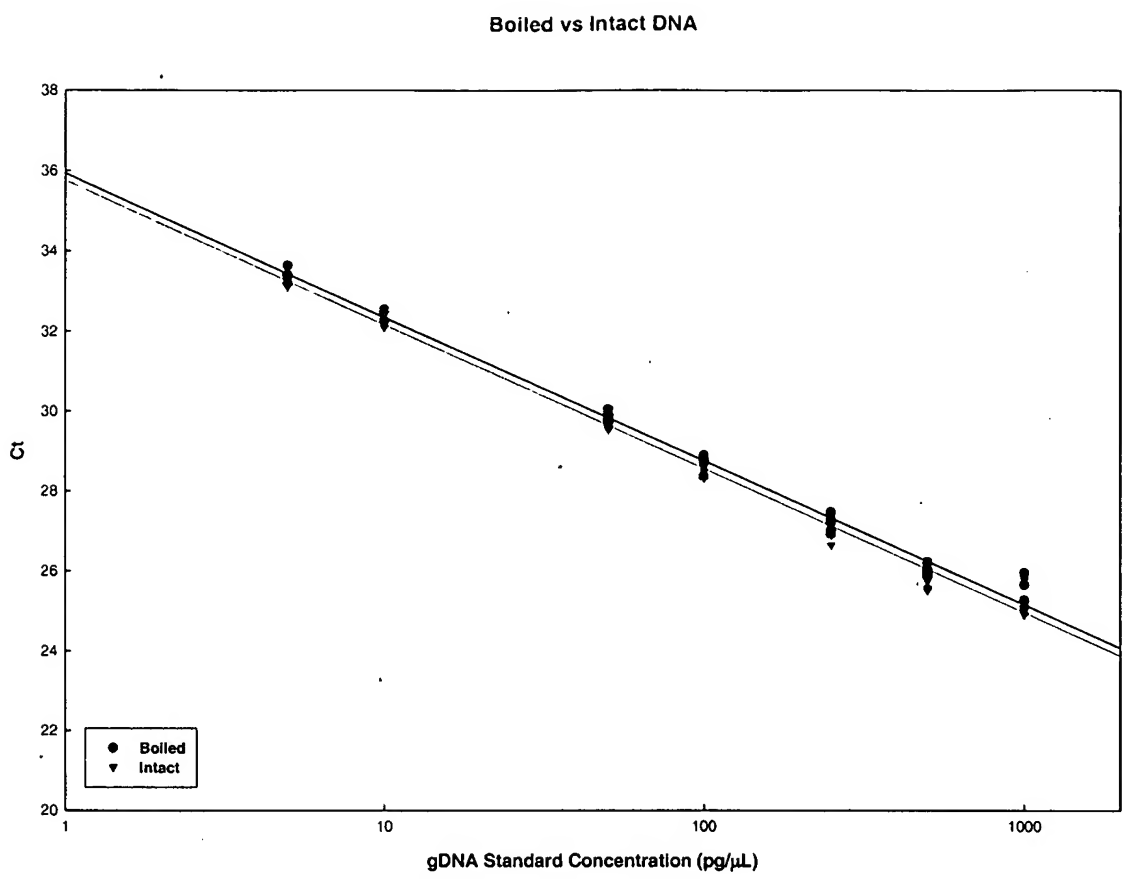
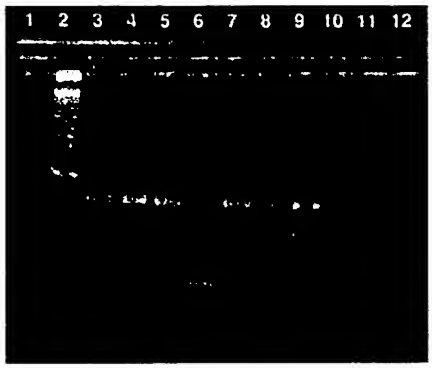

Boiled vs Intact DNA
38
36
34
32
30
28
26
24
22
20
Ct
Boiled
Intact
1
10
100
1000
gDNA Standard Concentration (pg/µL)

Figure 4

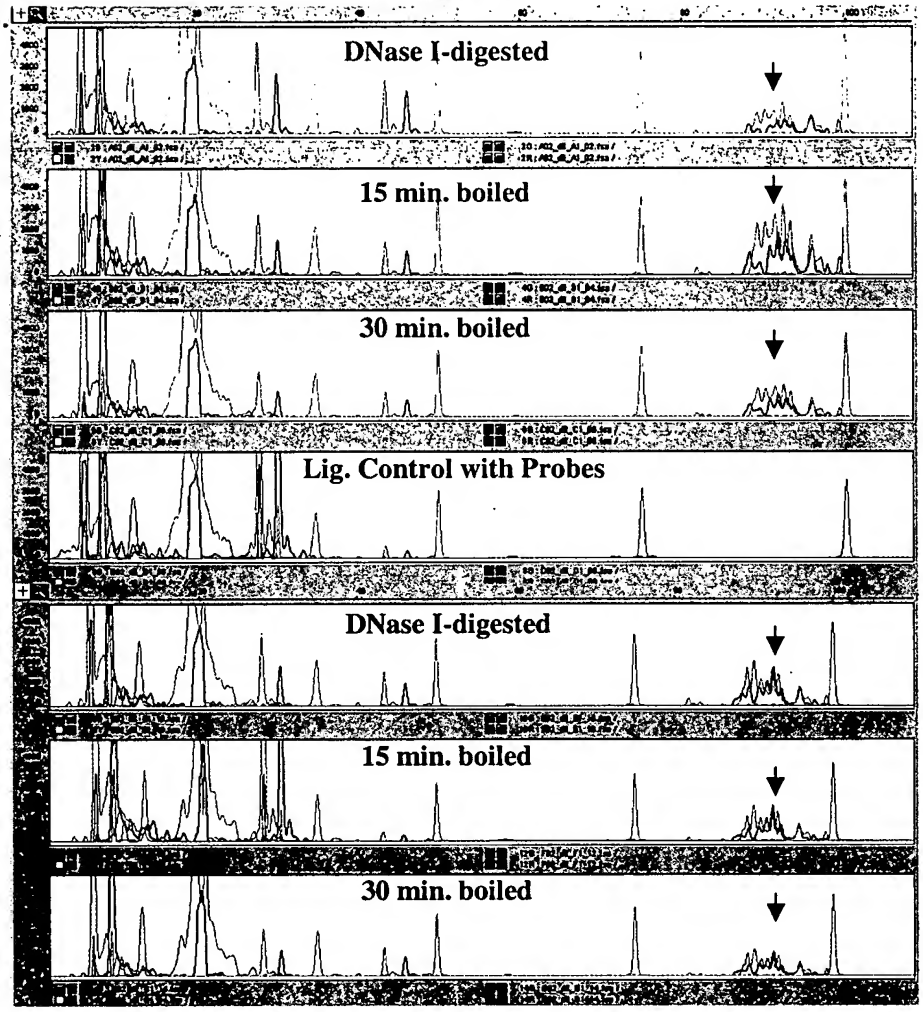
Boiled gDNA can Participate in OLA-PCR Reactions

A.



Lane 2: 25 bp marker
Lane 3: DNase I-digested CEPH 1347-2 gDNA
Lane 4: CEPH 1347-2 gDNA, boiled 15 min.
Lane 5: CEPH 1347-2 gDNA, boiled 30 min.
Lane 6: Ligation control (no gDNA)
Lane 7: DNase I-digested NA-12565 gDNA
Lane 8: NA-12565 gDNA, boiled 15 min.
Lane 9: NA-12565 gDNA, boiled 30 min.
Lane 10: Ligation control (no gDNA)

B.



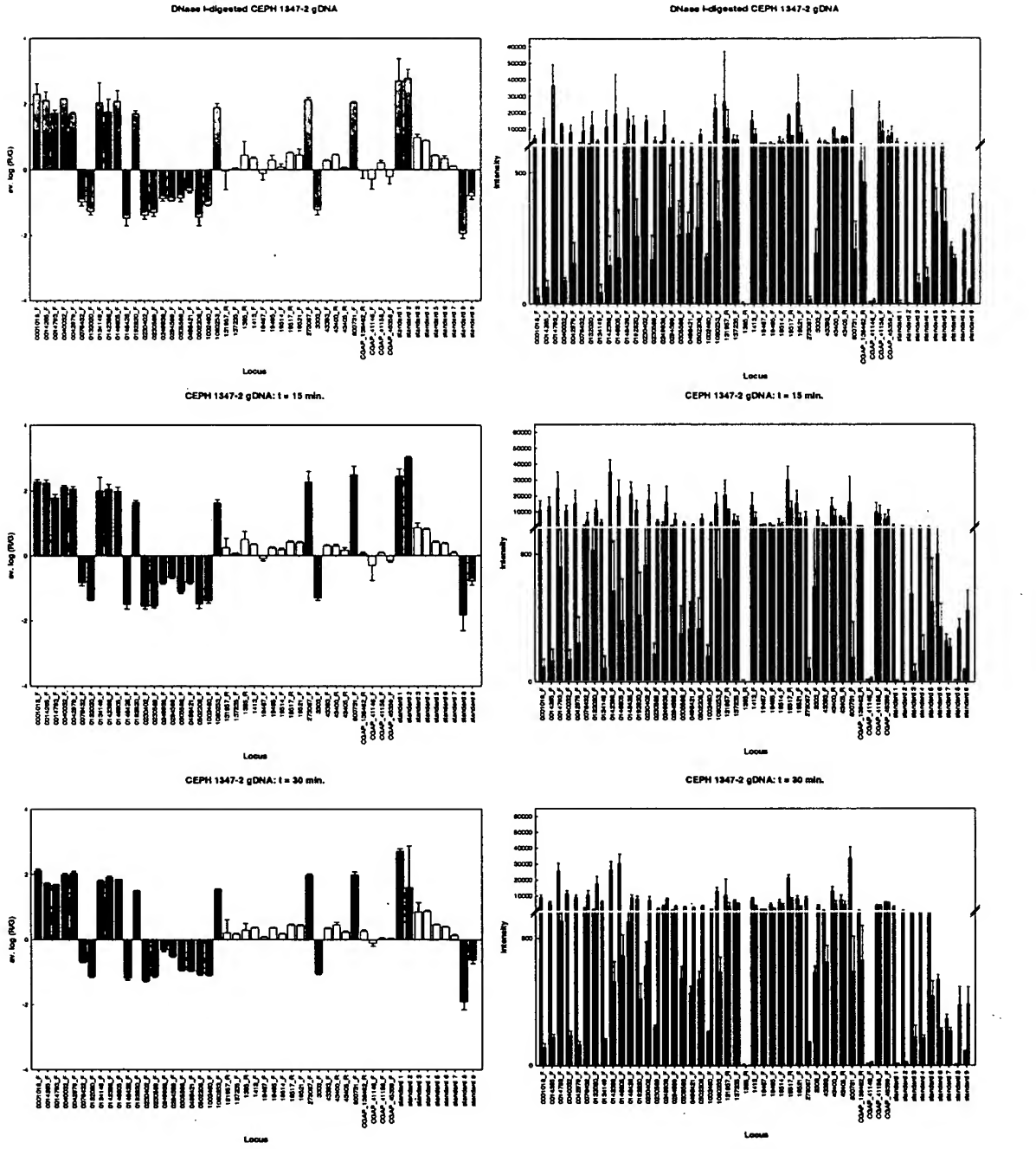
1347-2 gDNA

12565 gDNA

Boiled gDNA can be probed for specific SNPs by OLA-PCR. OLA-PCR reactions containing 10 ng/μL boiled CEPH 1347-2 or NA-12565 gDNA were probed for the presence of 40 specific SNPs. OLA-PCR products were detected by 4% agarose gel electrophoresis (A) or by capillary electrophoresis (B).

Figure 5

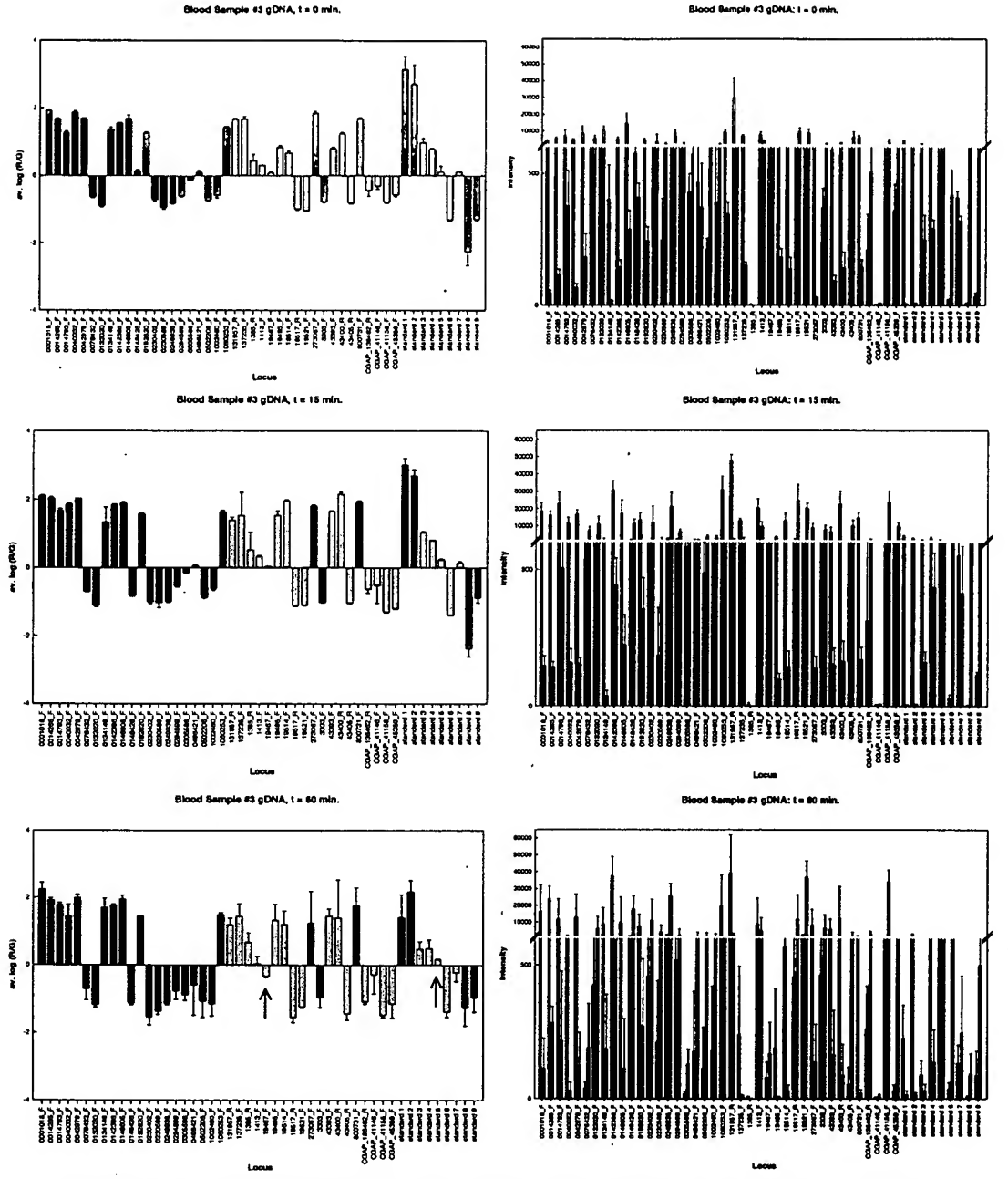
OLA-PCR Products Formed using Boiled gDNA Binds to PE-27 Arrays



OLA-PCR products generated using boiled (15 min. or 30 min. as specified) CEPH 1347-2 gDNA (set of bottom two panels) appear to bind similarly to PE-27 planar arrays as DNase I-digested CEPH 1347-2 gDNA (set of top panels). Similar average log R/G ratios (left panels) indicate similar genotype separation, while the slight changes in intensity was not reproducible (N= 2 experiments).

Figure 6

OLA-PCR Products Generated using Intact or Boiled gDNA Binds to PE-27 Arrays



OLA-PCR products generated using blood gDNA which was either unboiled (top set of panels) or boiled for 15 (middle panels), or 60 (bottom panels) min. bound to PE-27 planar arrays, indicating that either intact or low molecular weight fragments (~100-800 bp) of gDNA can be used to screen SNPs. However, the genotype separation data (left panels) obtained with the longer boiling time appeared to be a slightly more variable (see arrows), and the fluorescence intensity (right panels) of some SNPs appeared to be reduced.

Figure 7